nanoto

assessing the potential health hazards of nanotechnology

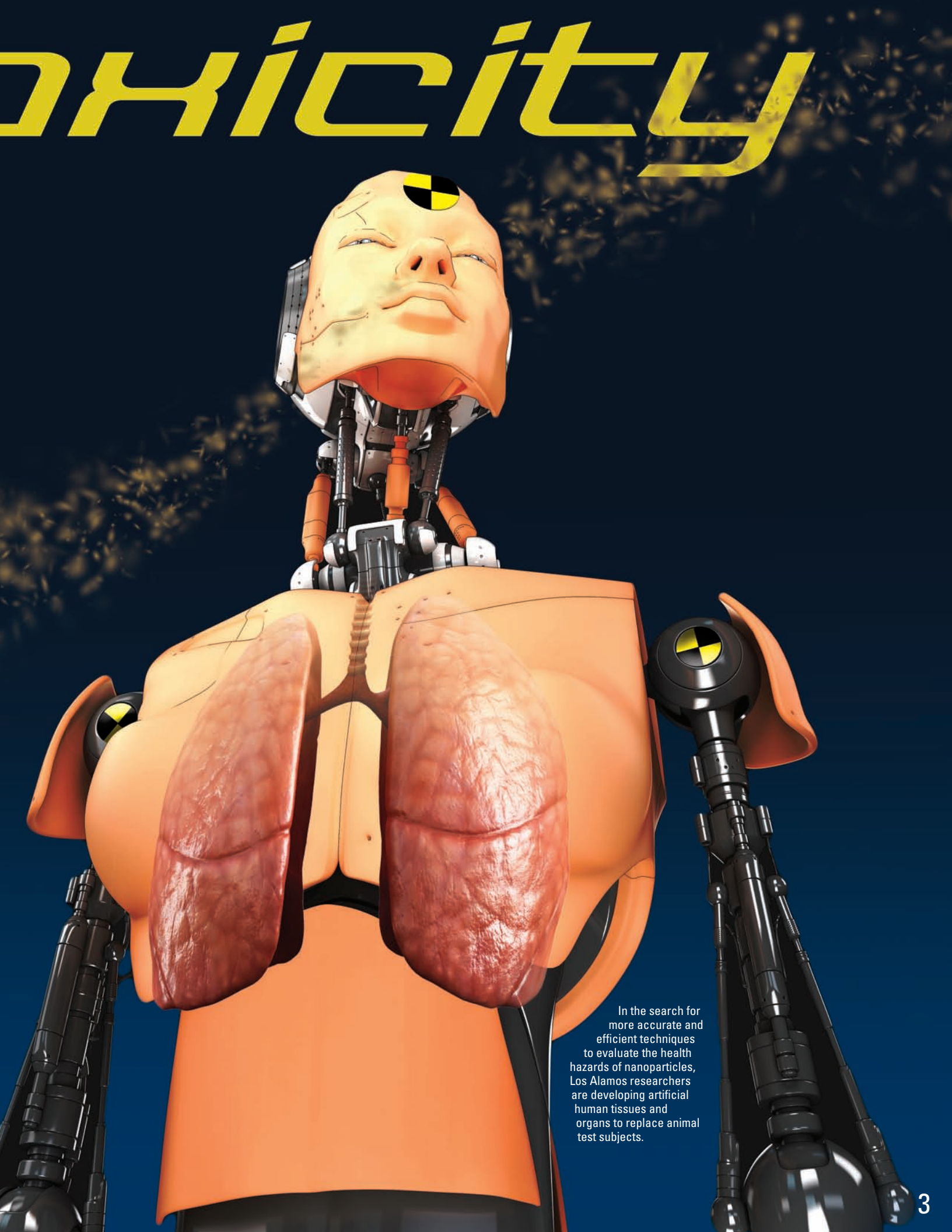
Used in products from sunscreens to solar panels, manufactured nanoparticles are proliferating so quickly that safety testing procedures are struggling to keep up. A revolutionary new approach to bioassessment using artificial human tissues may soon change that.

Asbestos was a popular 19th- and early 20th-century addition to building materials, insulation, and fire retardants. To this day, the mineral fibers still hide in walls and ceilings. With the danger of inhaling those tiny needles now well known, it's hard to imagine how asbestos became so ubiquitous.

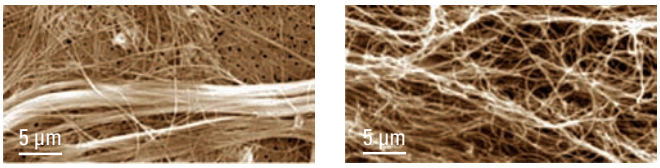
Now researchers at Los Alamos are among those applying the lessons learned from asbestos's legacy to even smaller particles that are building a modern-day industrial revolution. Nanoparticles come in a huge variety of shapes and chemical compositions, and their applications are equally varied, offering to revolutionize everything from energy production to disease treatment. Already, the little structures populate everyday items like fast food containers, cosmetics, sunscreens, and carbon-composite sports equipment.

As with any other chemical, producing and using engineered nanoparticles at the industrial scale raises questions about the risks of exposing people, like factory workers, who could touch or inhale them. Sorting out the toxicity of carbon tubes, copper spheres, cadmium discs, and roughly a thousand other commercially produced materials between the 1- and 100-nanometer scale hasn't kept pace with their rapidly evolving applications.

oxicity



In the search for more accurate and efficient techniques to evaluate the health hazards of nanoparticles, Los Alamos researchers are developing artificial human tissues and organs to replace animal test subjects.



Asbestos (left) and long, multi-walled carbon nanotubes (right) aren't just similar in appearance (shown here under a transmission electron microscope). In a 2008 U.K. study, mice exposed to carbon nanotubes developed inflammation similar to the lung disease caused by asbestos.

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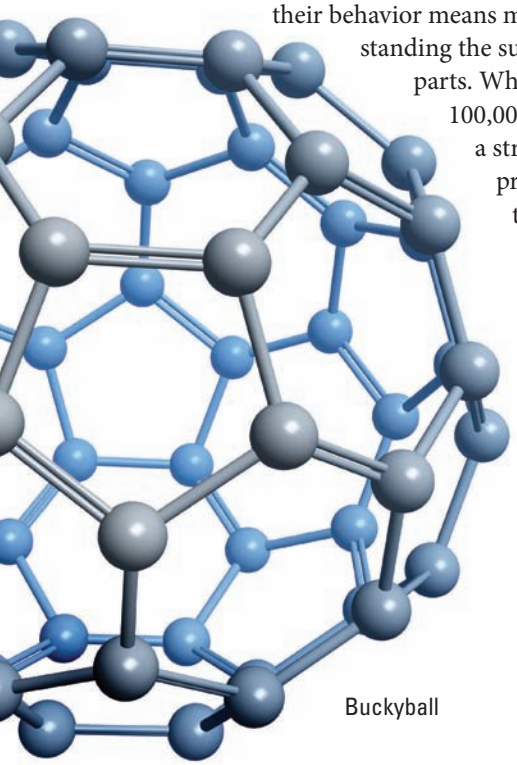
Fortunately, if researchers learn which properties separate a benign nanomaterial from an unsafe one, they can design materials to maximize functionality and minimize health impacts. Biologists and materials scientists at Los Alamos National Laboratory are working in the burgeoning field of nanotoxicology to uncover the harmful properties of tiny particles before the structures further permeate our material lives. Los Alamos scientist Rashi Iyer and her team are developing techniques to rapidly test and even predict which particles are the most damaging to lungs and skin. As they observe the biological impacts of nanomaterials, they are developing lab-grown human tissues and engineering synthetic millimeter-scale organs. In the future, those surrogates could replace animal testing with more relevant technology for evaluating not only the smallest engineered particles, but also many other chemicals, from pharmaceuticals to bioweapons. "We need a new paradigm for toxicity testing of anything, not just nanomaterials," says Iyer.

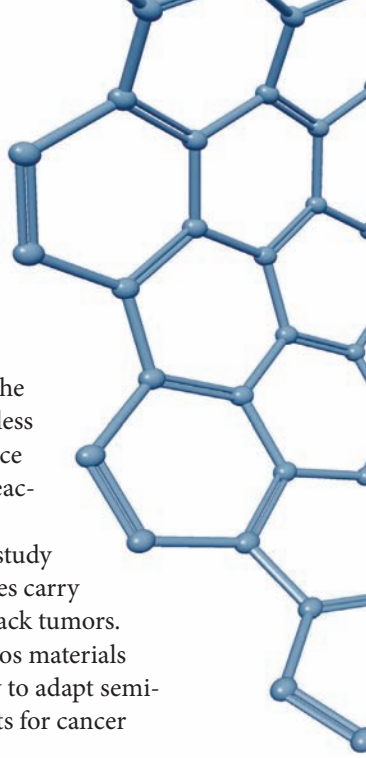
Sizing Up Nanoparticles

Molecular and atomic scale particles are built with elements from all parts of the periodic table, and predicting their behavior means more than understanding the sum of their molecular parts. When particles are about 100,000 times smaller than a strand of human hair, properties like conductivity, optical behavior, and chemical reactivity are different than those of a larger version of the same material. These differences within the same kinds of materials lend special uncertainty to nanoparticle toxicity.



Buckyball

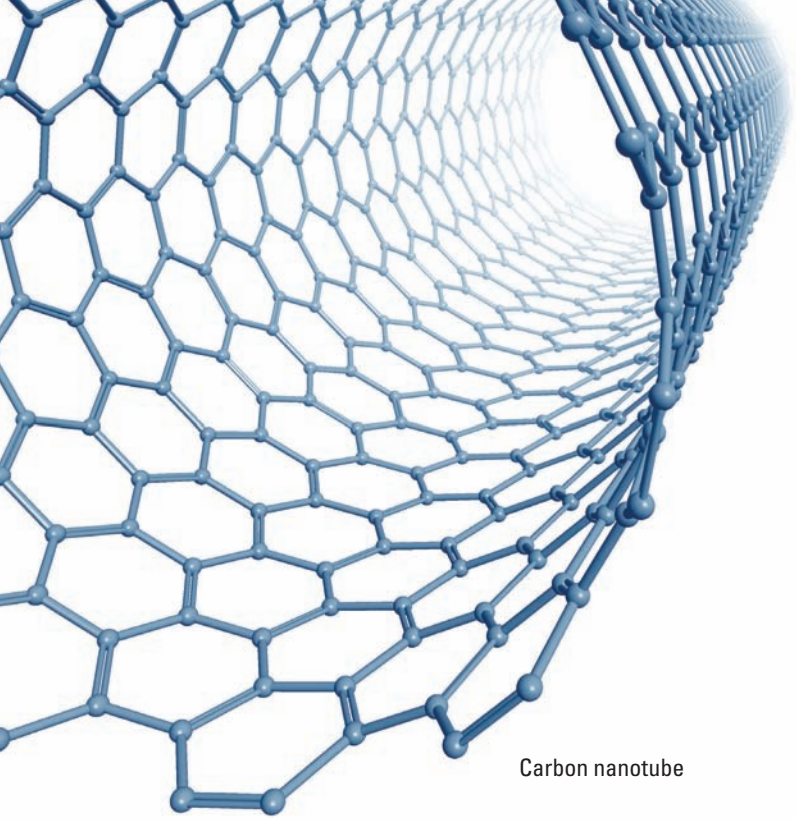
For example, small particle size translates into a higher surface area-to-volume ratio and enhances chemical reactivity, much like granulated sugar dissolving more rapidly than a sugar cube into tea. Gold, for example, is one of the most striking cases of the nanoscale boosting a material's reactivity. At less than 5 nanometers the typically inert substance becomes a catalyst that speeds up chemical reactions. Some chemists are leveraging the exaggerated surface area of gold nanoparticles to study potential cancer treatments where the particles carry therapeutic surface coatings and precisely attack tumors. Similarly, Iyer is collaborating with Los Alamos materials scientist Jennifer Hollingsworth to study how to adapt semiconducting nanoparticles called quantum dots for cancer therapy.



Unfortunately, the properties that make nanoscale materials valuable for biomedicine, or building the next generation of energy technologies, could come with a cost. Some nanoscale materials are more reactive with the body's proteins and DNA than their larger counterparts. If those risks go unrecognized, they could cause harm to human health, beginning at the cellular level.

A 2008 U.K. study was one of the first to address nanotoxicity in animals by investigating nanomaterials with a striking physical resemblance to asbestos. When researchers exposed laboratory mice to long, narrow carbon nanotube fibers, the mice developed tissue inflammation very similar to asbestosis—a chronic lung disease caused by asbestos. Curly or short nanotubes did not cause the same response, showing that nanoparticle toxicity can depend on properties like shape and size even when the chemical composition is the same.

Mentioning asbestos and nanoparticles in the same breath generates controversy, as some nanotechnologists warn that unwarranted panic over toxicity could stifle innovation. While there are no federal regulations specific to nanomaterials, Los Alamos and the Department of Energy have developed their own safety guidelines for worker and environmental protection (see "Nanosafety Starts Here," at right). Considering the breadth of nanotechnology research at Los Alamos, Iyer saw an opportunity to proactively understand nanotoxicology in parallel with the rapid discovery of new materials. "We're going to make these materials to address 21st century needs, like energy sustainability, but we need to understand their impact," she says.



Carbon nanotube

However, safer design won't happen by individually screening every single nanomaterial invented. Consider, for example, the variety of lengths, surface coatings, and manufacturing impurities for just the major kinds of single-walled carbon nanotubes (double-walled varieties also exist), and it's possible to generate more than 50,000 distinct samples just within that one category of nanomaterial. Toxicologists like Iyer could spend the rest of their lives doing nothing but testing nanotubes and hardly make a dent in the problem.

The dizzying array of nanoparticle features and the variety of ways researchers test their toxicity—from injecting live mice to exposing human cell cultures—is limiting how findings from researchers can aid industry or government agencies in developing nanomaterial safety policies. Testing each new nanostructure or its properties one at a time to identify the next asbestos amounts to looking for a nano-sized needle—or tube or wire—in a haystack. Iyer wants to streamline the haphazard nature of nanoparticle testing while developing guiding principles for materials scientists as they design and commercialize new particles.

She and others on her team are studying traditional toxicological responses like how particles affect cell growth, division, death, and metabolism. But they

nanosafety starts here

Los Alamos has been involved in nanotechnology research since the discipline's infancy. In 2006, the Center for Integrated Nanotechnologies (CINT) opened as part of the National Nanotechnology Initiative. Researchers from many institutions use joint CINT facilities at Los Alamos and Sandia National Laboratories to investigate all aspects of nanoscience and nanotechnology.

Part of the Los Alamos research is studying the toxicity of nanomaterials, while managing uncertainties about their risks to protect employees and the public. There are currently no state or federal regulations specifically addressing nanomaterials and no established recommendations for nanomaterial exposure levels. However, the National Institute for Occupational Safety and Health (NIOSH) and the Environmental Protection Agency also study nanotoxicity and provide safety recommendations.

The Department of Energy also created a formal order to provide guidelines for working with nanomaterials. Researchers and industrial hygiene professionals at Los Alamos and Sandia contributed, and the order is the foundation for nanomaterial safety policy at Los Alamos.

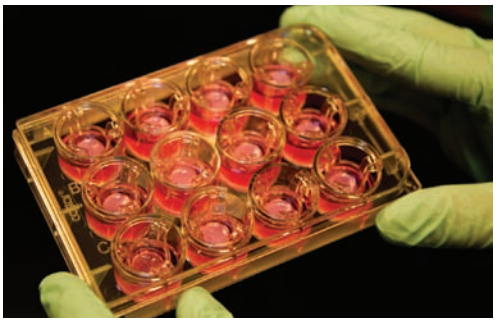
According to CINT Director David Morris, Los Alamos assumes that nanomaterials are at least as toxic as the bulk materials from which they are engineered. The Lab also recognizes that nanoparticles are potentially more toxic than the substances from which they are made. Understanding and predicting the extent of that toxicity is a major motivation for the collaboration between toxicologist Rashi Iyer and CINT scientist Jennifer Hollingsworth.

"No one appreciated that asbestos has toxicity above and beyond what the chemical constituents were until people started getting sick," says Morris. The Laboratory applies that lesson by minimizing researcher exposure to nanomaterials and keeping nanoparticles out of the environment.

For example, nanomaterial researchers at the Lab follow NIOSH recommendations and Los Alamos policies. Any potentially unbound nanomaterials, such as powders, in her lab are confined to chemical fume hoods or gloveboxes and stored in secure containers. The laboratory treats nanomaterial wastes as hazardous; they can't simply be poured down the drain.

"All the protections we have in place for working with carcinogenic or toxic chemicals are applied to nanomaterials," says Hollingsworth.

Los Alamos researchers work with very small nanomaterial quantities and take precautions while doing so. Their greatest concern is what happens when nanoparticles are made by the ton in industrial settings or in forms that could be inhaled. This is something the Los Alamos research aims to answer.



Human tissues for testing nanotoxicity are grown in quarter-sized wells that allow for rapid laboratory analysis.

are also finding early success with a new approach to the problem that drills down to the molecular level and explains how specific biomolecules within the cells of a tissue respond to nanoparticles.

A More Human Surrogate

Iyer and her colleagues learned that the field needed a more systematic approach when they observed in a 2010 study that even a small design tweak could influence a nanoparticle's toxicity. They exposed human skin and lung cells to molecules of buckminsterfullerene, a soccer ball-shaped cage of 60 carbon atoms configured like the geodesic domes of its namesake. Buckyballs, as they are sometimes called, are already manufactured in multi-ton quantities for use in sporting goods, such as lightweight tennis and badminton rackets, and are being tested as tiny vehicles for drug delivery.

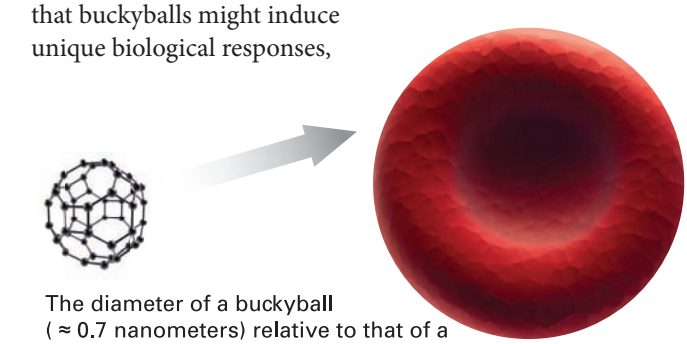
Iyer's chemistry colleague Hsing-Lin Wang selected three different buckyball variations: the standard pure carbon version and two with side chains—molecular adornments that are commonly attached to nanoparticles to change their behavior or function. When they exposed human skin cells to the buckyballs in the laboratory, one of the side chain varieties put cells in a kind of suspended animation. This process of senescence, where cells neither die nor divide, could cause organ dysfunction and eventually disease, but could also prevent cancerous tumors from expanding if scientists learn how to harness the effect.

The study was the first to find evidence that buckyballs might induce unique biological responses, including cellular aging. It also served as an important baseline for Iyer and her colleagues to learn how more complex tissues differ from single-cell layers when exposed to nanoparticles. Using single cell layers to mimic human exposure to a potential toxin, as in the buckyball study, is a common method in toxicology, but it's literally a one-dimensional approach. Monolayer cell cultures enable rapid testing but are a poor substitute for the diversity of interacting cells and chemical signals in real tissues and organs. For example, single-cell layers are immersed in liquid, but, as Iyer puts it, "we're not fish." Our own lung and skin tissues contact air on one side and fluid on the other, forming many cell types with defenses adapted for putting up a more concerted fight against invading nanostructures.

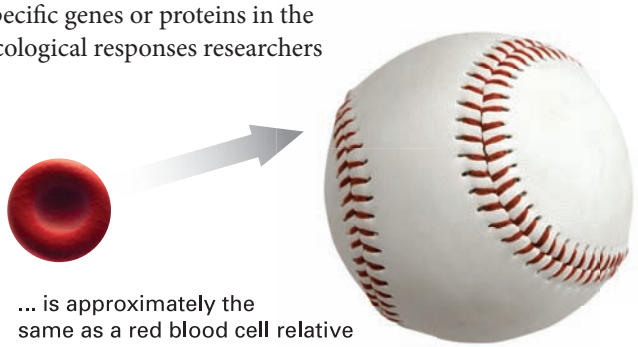
In addition to cell monolayers, non-human mammals like rats and mice are also common stand-ins for human testing. However, exposing them to nanoparticles is not just fraught with ethical dilemmas: their relevance to human toxicology is questionable, and their value is limited by the slow pace and expense of animal testing. A majority of the common chemicals we are exposed to daily have never been tested in animals. It's just not feasible, says Iyer. As animal welfare guidelines are beginning to encourage reduced animal use in research, animal testing's prominence in toxicology may wane.

For Iyer's team, a better mimic for exposure to nanoparticles is human lung and skin tissue constructed in the laboratory. Amber Nagy, a postdoctoral researcher in toxicology, came to Los Alamos specifically to work with Iyer to develop *in vitro* human lung tissue, which exists in a small dish outside of a human body.

While it is possible to order commercial lung tissue, making synthetic lung tissue is less expensive—less than 1/25th the cost—and gives the team more control over their experiments. The reduced expense alone is a compelling reason to develop in-house tissues, but there are significant scientific advantages as well. For example, commercial tissues can't be readily manipulated, while Iyer's group can use their own inventions to delete a particular gene or protein. These "knockout models" help explain the function of specific genes or proteins in the toxicological responses researchers



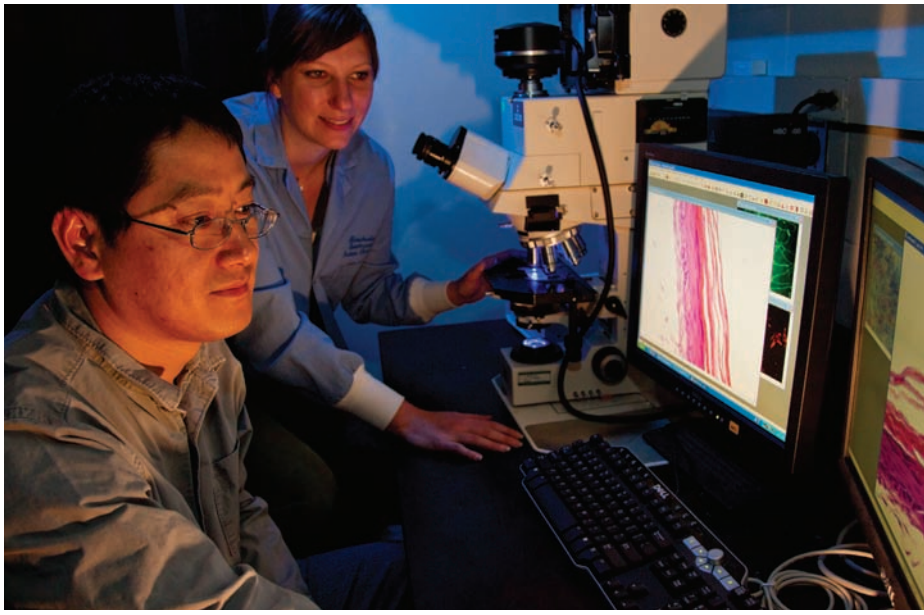
The diameter of a buckyball (≈ 0.7 nanometers) relative to that of a human red blood cell (≈ 8 micrometers) ...



... is approximately the same as a red blood cell relative to a baseball (≈ 7.5 centimeters) ...

observe and would complement Iyer's systematic approach for predicting how individuals will handle nanoparticle exposure.

Los Alamos toxicologist Jun Gao joined Iyer's nanotoxicity team in 2007 and has successfully grown a multi-layer human skin tissue, which Gao and Iyer are finding considerably more realistic than a traditional cell monolayer. If Iyer and her team learn how to control key variables, such as skin pigment expression or cell involvement in allergic responses, they could eventually perform human population studies in the laboratory without ever exposing people to potential toxins. Custom-grown tissues could eventually let them test how smokers and non-smokers or people with different ultraviolet exposure histories and skin pigments will respond to different nanoparticles.



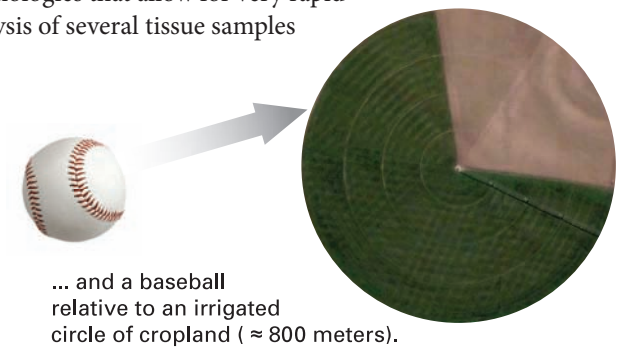
Los Alamos researchers Jun Gao (left) and Amber Nagy examine the lab-grown tissues they develop.

Developing human tissue in a tiny dish is more than simply creating a layer cake-like mixture of different cell types. Gao and Nagy must confirm that they have grown something that behaves like human tissue. To validate the function of their engineered human lung tissues, they turn to asbestos, a well-known lung toxin with decades of research explaining how it damages tissue. When exposed to crocidolite asbestos, one of the more hazardous varieties of the mineral, both natural and artificial tissues respond with inflammation, a decrease in mitochondrial metabolism, and a specific type of cell death. The tissues must also be the same down to the molecular level, or the resemblances are only superficial. Morphological markers such as gel-forming proteins called mucin, hair-like cell extensions called cilia, and tight junctions between cells, tell Nagy and Gao that they've made a genuine tissue mimic, not just a soup of cells in a tray.

Getting Charged Up

With tissues constructed, Iyer and her team are now working to predict which nanoparticles will be toxic and which will be harmless. Using cutting-edge technologies that allow for very rapid analysis of several tissue samples at once, they are able to monitor changes in protein- and gene-level characteristics of the nanomaterial. These "omic" technologies are coming to the forefront as ways to measure impacts on the entire collection of genes (the genome), proteins (the proteome), metabolites (the metabalome), or RNA (the transcriptome). With each particle they test on each cell type, Iyer's proteomics team, Srinivas Iyer and Tim Sanchez, generates data on how gene and protein regulation changes depend on a particular nanoparticle or its properties.

To analyze the gene transcription and protein expression data that could be linked to toxicity, they turn to Los Alamos computational biology colleagues Jeffrey Drocco and Jian Song. By using statistical tools to look at the data in a mathematically unbiased way, Drocco and Song find otherwise hidden relationships between toxicity and molecular responses. In the near future, understanding those relationships could enable the discovery and identification of nanomaterial-specific biomarkers to develop diagnostic tools that will determine past and current exposure to nanomaterials.



... and a baseball relative to an irrigated circle of cropland (≈ 800 meters).

Buckministerfullerene ("buckyball"), a spherical nanoparticle with 60 carbon atoms, is less than one nanometer in size—insidiously small relative to the cells of the human body. It's roughly 10,000 times smaller than a red blood cell, which is comparable to the difference between a baseball and an irrigated circle of cropland that's easily visible from an airplane.

If Iyer and her team learn how to control key variables ... they could eventually perform human population studies in the laboratory ***without ever exposing people to potential toxins.***

The approach has yielded results that the toxicologists would have missed on their own. Through statistical sleuthing, Song found that when Iyer's team exposed skin cells to fullerenes, the genes perturbed were associated with heart toxicity. "You don't really have to use heart cells to figure out if something is a heart toxicant," says Iyer. "Down the line, the idea is that if you have 10,000 nanomaterials and you look at the molecular level response you should be able to *predict* toxicity."

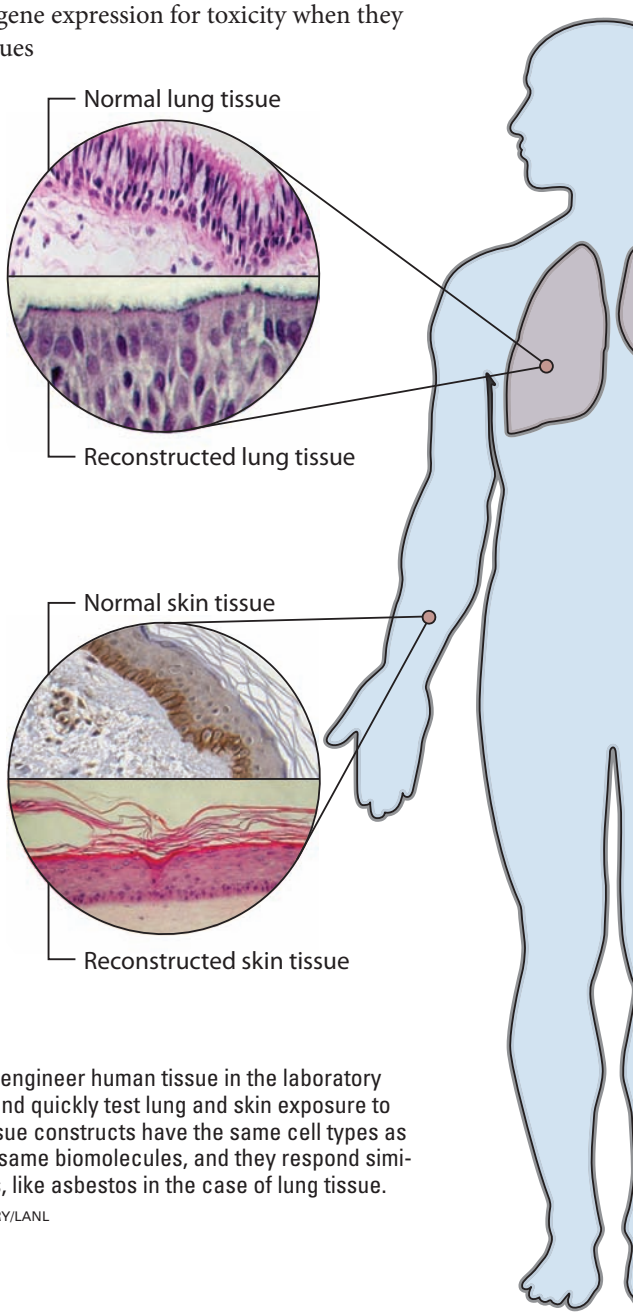
Iyer and her team first tested their molecular approach in a pilot study published last year. They exposed human lung cells to semiconducting nanocrystals engineered by Jennifer Hollingsworth, who designs materials for energy efficiency and biomedical applications as part of Los Alamos National Laboratory's Center for Integrative Nanotechnologies. Called quantum dots, the particles have optical and electronic properties that can be fine-tuned by changing their size, making them promising light emitters in energy-efficient LED lighting. Their size-dependent fluorescent colors are also ideal for staining and imaging live cells in exquisite detail and have potential application for detecting and treating cancer.

Much like radiation used in cancer treatment, quantum dots could be life-saving but could cause harm through unplanned exposure. As quantum dots are mass-produced for applications like LEDs, solar cells, and medicine, Hollingsworth wants to help bring toxicological knowledge into her designs. "I'm involved because I want to design materials that will either have a minimal biological impact or a predictable one," she says.

Iyer's group is already making some generalizations that could help Hollingsworth with that goal. When they used a variety of quantum dots to treat a single layer of cells that line the lung's bronchia, they found that positively charged dots were much more deadly to cells than their negatively charged counterparts, regardless of the length of side chains attached to the dots or their overall size. In fact, these materials perturbed distinct suites of genes, proteins, and pathways that might be due to the specific charge on the nanomaterial surface. The team has learned that negatively charged quantum dots and those with short side chains were the least toxic and could be the best option for medical applications. However, even in quantum dots that appear benign by traditional measures of toxicity, molecular-level data revealed that they increase gene expression associated with DNA damage.

The toxicity of positively charged nanoparticles isn't simply an aberration of quantum dots. Through their molecular data, the team sees a correlation between positive particle charge and gene expression for toxicity when they expose cells and tissues of lung and skin to many kinds of nanomaterials, including carbon nanotubes and buckyballs. When Drocco analyzes the data, the team notices that positively charged particles disrupt transcription, the first step of gene expression.

"It's not by chance," says Nagy. "We're able to separate the signal from the noise and see that there's a clear transcriptional response induced by nanomaterials of different charges. We don't



Los Alamos scientists engineer human tissue in the laboratory to realistically mimic and quickly test lung and skin exposure to nanoparticles. The tissue constructs have the same cell types as human tissue and the same biomolecules, and they respond similarly to known irritants, like asbestos in the case of lung tissue.

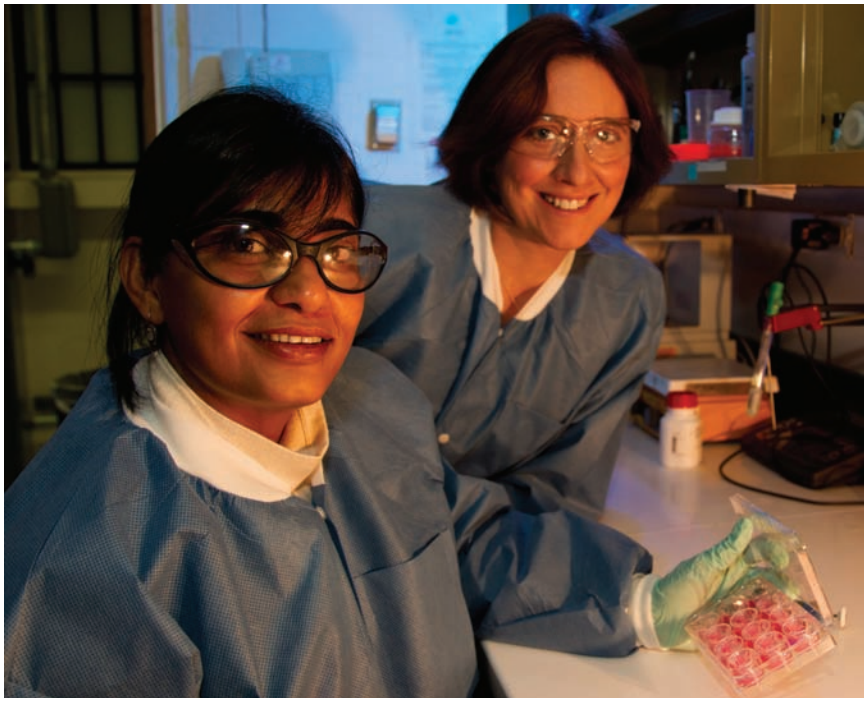
CREDIT: RASHI IYER LABORATORY/LANL

want a positively charged anything—unless we are trying to kill the cell—because it seems to induce more perturbation."

When the team exposes lung and skin tissues to nanoparticles, they see patterns of toxicity that mirror single cell layers treated with nanoparticles. Charge, side chain selection, and particle size are all factors in harming both cells and tissues. But with tissues, the cellular and molecular responses are much less severe (or even nonexistent) than those for the same dose applied to cells.

In lung tissues, protective mucous and tiny waving arms called cilia trap invading particles and move them away from the tissue, but those defenses are absent in single cell layers. "We've found that once you add structural complexity and different cell types, the biological response we observe is actually from all those different cell types in the tissue, not just the one type of cell," says Gao.

They're also using the complexity of tissue constructs to learn which nanoparticles are most likely to move through skin and circulate to other organs. Gao is imaging particles as they've lodged in different layers of skin. For example, he found that negatively charged quantum dots penetrate skin tissue while positively charged dots sever the tissue without much penetration. The results will assist with nanomaterial design for targeted drug delivery and determine which particles completely pass through several layers of human skin tissue.



Toxicologist Rashi Iyer (left) and materials scientist Jennifer Hollingsworth collaborate to predict and mitigate the toxicity of nanoparticles before they are mass produced.

Bringing *In Vitro* to Life

While toxicologists can't experiment with how a real human body responds to rogue nanoparticles or those used for medical purposes, Iyer is leading an effort to build the next best thing—a laboratory device that simulates human physiology and chemistry at 1/1000th the scale of a human body.

This system of engineered human organs—the Advanced Tissue-engineered Human Ectypal Network Analyzer (ATHENA), as it is named—will rapidly screen for the safety of nanoparticles and pharmaceuticals without the need of animal exposure studies. Moreover, Iyer and her colleagues are working toward developing ATHENA to study human exposure to biological weapons and test possible medical countermeasures.

In addition to overseeing the unification of an artificial heart, lung, liver, kidney, and arterial and venous systems with artificial blood, Iyer's group will breathe life into ATHENA by mimicking the bronchial and alveolar architecture of the human lung. The idea is to develop a platform that enables organ-to-organ communication to simulate the human body's response to any pathogen, material, drug, or chemical. Then, to take into account the physiological responses of organs that are not represented in ATHENA, Iyer has added placeholders for missing organs that will simulate the organ of choice, depending upon the nature of the investigation. "This is really the first time that a system of such magnitude and complexity will be developed, with the potential to eventually replace animal and human clinical studies," says Iyer.

Iyer isn't alone in her ambition to someday replace animal studies with technology that simulates our relationship with the materials we invent. The National Institutes of Health and the Defense Advanced Research Projects Agency are behind similar "human-on a-chip" technologies, funding separate teams to develop the next generation of bioassessment platforms. "It's a race to build a good version of Frankenstein's monster," she says, quickly adding that her engineered human will benefit society in a way the fictional one never could. ❖ LDRD

—Sarah Keller